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Studies on the threonine requirement in growing pigs

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

cor.	corrected
AA	Amino acids
ADF	Acid detergent fibre
ADL	Acid detergent lignin
BW	Body weigth
$BW^{0,75}$	Metabolic body weight
C	Control diet
CF	Crude fibre
CP	Crude protein
dig.	digestible
DM	Dry matter
$EAAL_B$	Basal endogenous amino acid losses
$EAAL_S$	Specific endogenous amino acid losses
Exp.	Experiment
GE	Gross energy
ME	Metabolizable energy
MJ	Megajoule
OM	Organic matter
N	Nitrogen
NDF	Neutral detergent fibre
P	Probability (level of significance)
pcd.	Precaecal digestible
ret	Retention
RMSE	Root mean square error
SD	Standard deviation
SE	Standard error
SEM	Standard error of means
SID	Standardised pc. amino acid digestibility
thr	Threonine

1 GENERAL INTRODUCTION

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1.1 BACKGROUND

Protein is besides carbohydrate and fat one of the most fundamental nutrients in a diet for pigs. In this respect it is important to know the protein or amino acid (AA) requirement of the pig, especially for the essential AA lysine, threonine, methionine and tryptophan. Many studies have been carried out to determine the AA requirement for pigs at different stages of growth, but the results show a considerable range, e.g. for tryptophan (Susenbeth, 2006). Furthermore there are several factors which influence the AA requirement of the pig like feed properties, level of growth or body weight. A considerable part of the AA requirement for maintenance is caused by endogenous losses (EAAL) in the gastro-intestinal tract. Factors affecting the amount of EAAL are feed intake, body weight, anti-nutritional factors of feeds, feed processing, protein, fat and fibre in the diet (Souffrant, 2001). Due to an increasing demand for human food and food prices as well as the increasing production of bioethanol, alternative low-cost feedstuffs have to be used predominantly fibre-rich by-products of food processing. However fibre-rich diets increase the EAAL (Schulze et. al., 1994) and consequently the AA requirement. Threonine is especially important in this context, because threonine concentration in endogenous protein is high, and therefore an increase in EAAL strongly affects the available amount of threonine for protein deposition.

1.2 ENDOGENOUS CRUDE PROTEIN AND AMINO ACID LOSSES

Endogenous crude protein can be defined as the amount of crude protein in faeces, which does not originate from the diet (Souffrant, 1991). Endogenous proteins originate from salivary, gastric, pancreatic, bile and small intestinal secretions, as well as mucus, sloughed epithelial cells and microbial protein (Jansman et al., 2002). It is assumed that only 20 to 30 % of the endogenous protein secreted or present in the lumen of the gastrointestinal tract reaches the end of the small intestine. Most of it is hydrolysed and absorbed before reaching the terminal ileum. This process is described as recycling of endogenous protein (Souffrant, 1991). As illustrated in Figure 1.1, EAAL can be divided into a basal (non-specific) and a specific fraction. The basal endogenous losses ($EAAL_B$) represent the protein and AA losses independent of diet composition. They are related to the amount of dry matter intake and are not influenced by diet composition. In contrast, the specific endogenous losses ($EAAL_S$) are

depending on the content of dietary fibre and the presence of anti-nutritional factors like tannins or lectins (Mosenthin and Sauer, 2000).

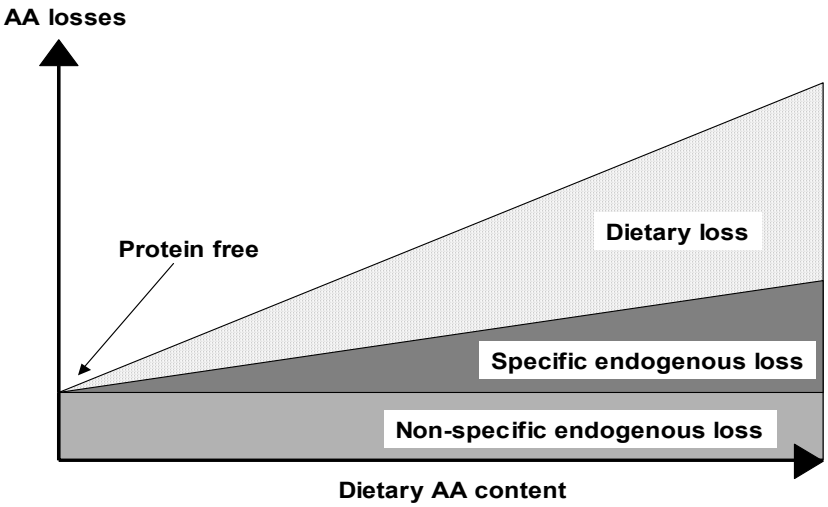

Figure 1.1 Sources of amino acid losses in ileal digesta (Mosenthin and Rademacher, 2003)

Several methods exist for the measurement of $EAAL_B$, but there is still no routine procedure to determine $EAAL_S$. The determination of $EAAL_S$ is only possible experimentally with tracers. The two most common methods for the measurement of $EAAL_B$ are the protein-free diet and the use of diets with highly digestible protein sources (casein or wheat gluten). The problem of the protein-free diet is that this method does not use physiological conditions and probably due to that reason $EAAL_B$ might be underestimated (De Lange et al., 1989). Other methods are the regression method, the enzymatically hydrolysed casein method (EHC) and the N-free diets with parenteral infusion of AA (Jansman et al., 2002).

Due to economical and ecological reasons it is very important to avoid protein excess or undersupply. The knowledge of $EAAL_B$ is one key information. If the $EAAL_B$ is not considered, the AA availability would be underestimated, because $EAAL_B$ are taken as undigested AA. There is general agreement in the literature that apparent precaecal (pc.) digestibility should be corrected for $EAAL_B$ to receive standardised pc. digestibility values (SID). The transformation of apparent pc. AA digestibility into standardised pc. digestibility values by correcting for $EAAL_B$ is described by the following equation (Mosenthin, 2003):

SID (%) = ((CP or AA intake - (pcd. CP or AA excretion – CP or $EAAL_B$)) / CP or AA intake) x 100

Corrections of apparent pc. digestibility values for both specific and basal protein and AA losses allow for the calculation of the true pc. crude protein and AA digestibility coefficients, as illustrated in Figure 1.2.

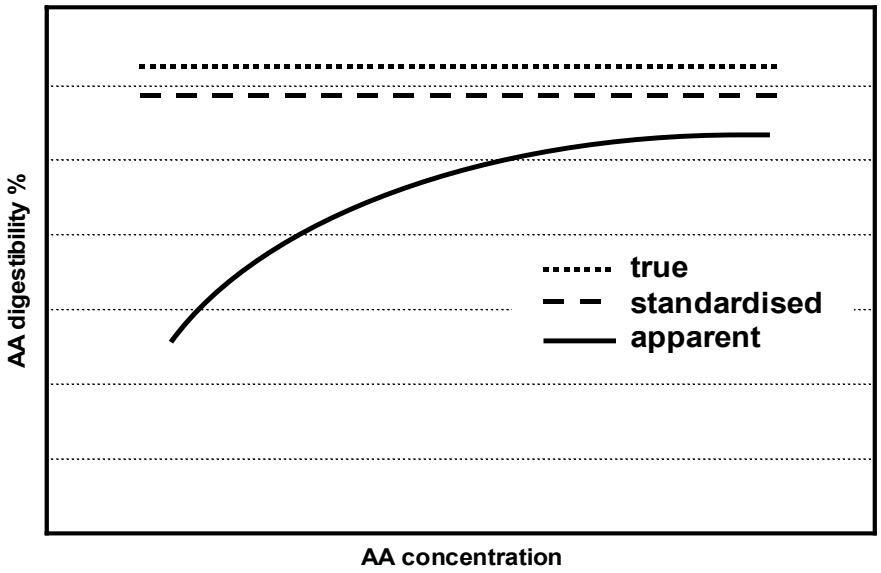

Figure 1.2 Diagram of the grades of the amino acid (AA) digestibility (Flachowsky, 2002; modified)

1.3 DIETARY FIBRE

Dietary fibre is the herbal cell wall or the storage of non-starch polysaccharides and lignin (Bach Knudsen, 2001), which are for the most part resistant to hydrolysis by the digestive enzymes of monogastric animals. The fractions of the dietary fibre are lignin, cellulose, hemicellulose, pectine, pentosane, beta-glucanes and parts of the oligosaccharides (Jeroch, 1999) (Figure 1.3). The cell wall fraction is very complex, which makes an accurate and complete analysis of cell walls in feedstuffs difficult. The three methods of fibre characterisation generally applied are: Weende crude fibre (Henneberg and Strohmann, 1859), detergent fibre (van Soest, 1973), and total dietary fibre (TDF). In the Weende method crude fibre is measured, which however includes only a part of the fibre. According to the van Soest method NDF, ADF and ADL are measured by boiling feed samples with different detergent solutions. The NDF includes hemicellulose, cellulose and lignin, and the ADF includes cellulose and lignin. TDF is more often used for human diets, but it has been used in a few pig nutrition studies as well. A schema of these three methods of fibre characterisation is given in Figure 1.3.

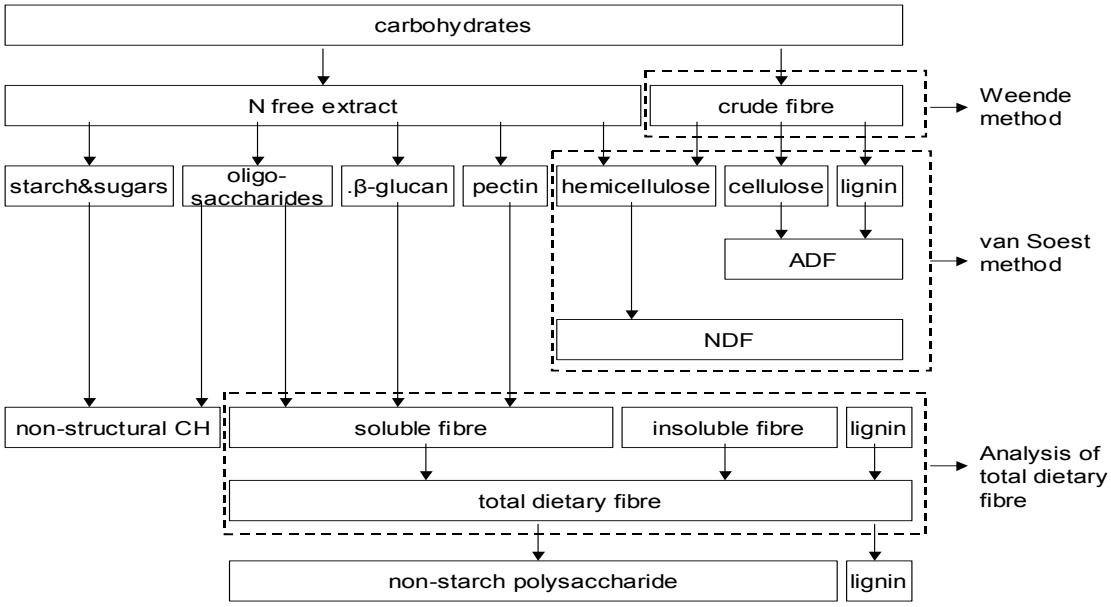

Figure 1.3 Three methods of fibre characterisation (Degen, 2006; modified)

Figure 1.3 clearly shows that in the detergent method hemicellulose, cellulose and lignin are measured, while the Weende crude fibre method gives a measure for cell wall content somewhere between the neutral detergent fibre and acid detergent fibre fraction. So in the Weende crude fibre method the crude fibre will be underestimated and for this reason the method of van Soest is more accurate.

There are several reasons for the increased interest in recent years in the use of fibrous ingredients for pig feeds. Because of the increasing demand for food with a low fibre content, there is an increased availability of high fibre by-products (Noblet and Le Goff, 2001). Furthermore there are positive effects of fibrous ingredients in pig feeds, those on animal welfare (Ramonet et al., 1999) or ammonia emission (Canh et al., 1998).

1.4 EFFECT OF DIETARY FIBRE ON ILEAL ENDOGENOUS CRUDE PROTEIN AND AMINO ACID LOSSES AND ON PROTEIN DIGESTIBILITY

Generally non-starch polysaccharides may affect many processes in the gastrointestinal tract. They affect the production and activity of digestive enzymes, intestinal morphology, the microbial population in various parts of the gut, and the secretion of certain hormones (de Lange, 2000).

It is demonstrated that dietary fibre enhances the EAAL either by enhancing the excretion itself and/or by reducing its re-absorption (Schulze et al, 1994). Consequently, protein in fibre

rich diets may show higher variations in apparent and true pc. digestibility. From the literature it is evident that pc. digestibility of protein is depressed in the presence of dietary fibre. It is difficult to describe quantitatively the effects of various fibre components on digestibility and EAAL, because they are not homogenous. Furthermore, there are interactions between different nutrients. The level and the source of dietary fibre are the two most important factors influencing the amount of EAAL (Sauer and Ozimek, 1986). Table 1.1 shows the effect of fibre in the diet on the endogenous nitrogen losses (Schulze et al., 1994).

Table 1.1 Differentiation of ileal nitrogen (N) losses (g/d) into additional total N, additional exogenous N and additional non-NDF-N excretion of N as affected by various levels of purified NDF in the diets of barrows (Schulze et al., 1994; modified)

	Purified NDF					
Item	0	60	120	180	SEM	P-value
Ileal N g/d						
Total excretion	2.787	3.041	3.563	4.129	0.152	0.001
additional total N	-	0.255	0.776	1.342	0.169	0.001
additional exogenous N	-	0.198	0.369	0.518	0.010	0.001
additional non-NDF-N	-	0.056	0.407	0.824	0.162	0.006

1.5 THREONINE AS FIRST LIMITING AMINO ACID

Threonine is an alpha-AA with the chemical formula $C_4H_8NO_3$ (Figure 1.4). This essential AA is classified as polar. Together with serine and tyrosine, threonine is one of three proteinogenic AA bearing an alkohol group. The threonine residue is susceptible to numerous posttranslational modifications. The hydroxy side chain can undergo O-linked glycosylation. Additionally, threonine residues undergo phosphorylation through the action of a threonine kinase.

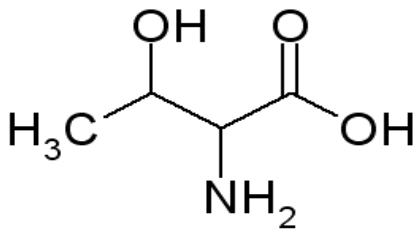

Figure 1.4 Chemical structure of threonine

In nutrition beside the energy and nutrient supply the fulfilment of demand of AA is very important. Typically lysine is the first-limiting AA in diets for pigs. Therefore, the threonine requirement is often related to that of lysine and its adequate supply as well as for other essential AA derived from the composition of the ideal protein. But also the other AA can be

first-limiting. Especially in the recent years the use of fibrous ingredients for pig feeds has significantly increased. The diversification of feed supply for pigs has lead to additional limitations in the availability of some essential AA (e.g. threonine), which under certain circumstances may become limiting (Henry and Seve, 1993). This is interesting in the context to EAAL, because of the high threonine content of endogenous protein (Bikker et al., 2007). In Table 1.2 mean basal endogenous protein and AA losses are given.

Table 1.2 Mean basal endogenous crude protein (CP) and amino acid (AA) losses (g/kg DM intake) (Jansman et al., 1990; modified)

CP and AA	CP	Lys	Met+Cys	Thr	Try	Isol	Leu	Val	Hist	Arg	Phe
Endogenous losses (g/kg DM intake)	11.8	0.40	0.32	0.61	0.14	0.38	0.49	0.54	0.19	0.39	0.34

DM: dry matter, Lys: lysine, Met+Cys: methionine+cystine, Thr: threonine, Try: tryptophan, Iso: isoleucin, Leu: leucine, Val: valin, Hist: histidin, Arg: arginine, Phe: phenylalanine

1.6 SCOPE AND OBJECTIVE OF THE THESIS

Dietary factors (e. g. fibre) which may affect endogenous protein/threonine losses and may increase AA maintenance requirement are usually not considered in feeding standards (GfE, 2006; NRC, 1998). There is evidence from the literature that N retention in the animal is reduced by adding fibre to a threonine limiting diet, and it can be stated that this reduction is caused by increased EAAL (Schulze et. al., 1994). However, only few studies on the extent of this negative effect are available. Therefore, the aim of the studies which are presented in this thesis is the determination of the negative effects of dietary fibre and to quantify the threonine requirement in pigs as a function of the fibre concentration.

In Chapter 2, the effect of the dietary fibre on N retention of the animals and on fibre associated threonine losses were determined in an extensive balance study in growing pigs. Because the classical experimental approach with cannulated animals might have lead to some technical problems in fibre rich diets and shows considerable limitations in determination of the overall effect of fibre on the amount of AA available for growth, an alternative indirect approach was used in the present study, where the reduction of N retention caused by fibre supplementation to a threonine limiting diet was taken as an estimate for increased endogenous threonine losses. Furthermore, it was tested whether the extent of this effect depends on the fibre source and/or fibre concentration.

In Chapter 3, all available data on the optimum dietary threonine : lysine ratio ($T:L_{opt}$) for growing pigs reported in the literature are summarized and evaluated. Furthermore, the attempt is undertaken to identify possible factors responsible for the variation in $T:L_{opt}$. Special attention is paid to the effect of fibre on $T:L_{opt}$.

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2 EFFECT OF DIETARY FIBRE ON NITROGEN RETENTION AND FIBRE ASSOCIATED THREONINE LOSSES IN GROWING PIGS

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2.1 ABSTRACT

Dietary fibre is discussed to act as an anti-nutritional factor by reducing apparent precaecal protein and amino acid (AA) digestibility due to reduced absorption and/or increased endogenous secretion. However, the amounts of protein and AA of endogenous origin appearing at the terminal ileum as determined in cannulated animals do not represent the total amount of losses associated with endogenous secretion. A high proportion of secreted protein is reabsorbed and does not reach the terminal ileum, and losses occur during synthesis of endogenous protein. Therefore, in the present study an alternative indirect approach was used, where the reduction of nitrogen (N) retention in a threonine (thr) limiting diet was taken as a sensitive indicator for fibre associated thr losses. Two experiments were conducted with twelve castrated male pigs each between 37 and 75 kg of body weight (BW) to measure the effect of thr intake and the effect of 150 and 300 g/d fibre intake from wheat bran (WBF) (Exp.1), and of 150 g/d fibre from rape seed (RSF), cassava leaves (CLF), and cassava roots (CRF) (Exp. 2) on N retention, respectively. Two balance periods were performed in Exp. 1, and three periods in Exp. 2 where animals were subjected to the dietary treatments according to a cross-over design. All animals received 1.35 kg/d of a basal diet which consisted (g/kg) of 742 wheat, 70 soybean meal, 50 wheat bran, 60 wheat gluten, 40 mineral-vitamin-premix, 30 soybean oil, and free AA to ensure thr being the first-limiting AA. To determine the effect of thr on N retention, the intake of the basal diet was reduced to 1.15 kg/d and supplemented with corn starch to reach equal energy intake and an unchanged AA pattern. With increasing BW additional starch was added to all diets to ensure a constant energy intake of 1.25 MJ ME/kg $BW^{0.75}$. Since the fibre sources contained small amounts of thr, N retentions were corrected for pcd. thr intake originating from the fibre sources according to the thr effect on N retention as determined in Exp. 1. Corrected N retentions were affected by fibre level ($p=0.007$) and source ($p<0.001$). Fibre associated thr losses amounted for 3.2, 3.3, 3.4, 1.2, and 1.1 g/kg WBF (150), WBF (300), RSF, CLF, and CRF, respectively. It can be concluded that thr losses per g dietary fibre depend on the fibre source and are not affected by their concentration in the diet.

KEYWORDS: Pig, Dietary Fibre, Threonine, N Retention, Endogenous Losses

2.2 INTRODUCTION

The need for using fibre rich by-products of food production in pig feeding has significantly increased in recent years, since the use of high quality feeds compete with a globally increasing demand for human food as well as for bioenergy production. Fibre contributes to a significant proportion to the energy value in many feedstuffs, e.g. to 0.28, 0.29, and 0.84 in rapeseed meal, wheat bran, and sugar beet pulp (acc. to feed table of DLG, 1991 and GfE, 2008). However, dietary fibre may reduce apparent protein and AA digestibility and/or increase EAAL (Schulze et. al., 1994) by increased sloughing of intestinal mucosal cells and enhanced mucus production (Sauer et al., 1991). Increased secretion and reduced reabsorption of endogenous N is affected by the level and the source of fibre (Schulze et al., 1995). This necessitates an increase in the content of protein and AA in fibre rich diets to meet the demand for growth and protein deposition of the animals. In general, the AA requirements for maintenance, of which EAAL account for a high proportion (Wang et al., 1989), have been related to BW (GfE, 2008; NRC, 1998). However dietary factors (e.g. feed intake level, fibre and other anti-nutritional factors) which also affect EAAL (Souffrant, 2001) and consequently maintenance requirement are not considered. Therefore, the objective of this study was to quantify the anti-nutritional effects of different types of dietary fibre which may lead to an increased AA requirement for maintenance. The experimental approach generally applied to quantify EAAL is to determine the respective amounts at the end of the ileum in cannulated animals (Sauer et al., 2000). This experimental approach shows technical problems in high fibre diets (congestion of intestinal fistula; Sauer et al., 1992). Furthermore, the amounts of protein and AA of endogenous origin at the terminal ileum do not reflect the total amount of losses associated with endogenous secretion. A high proportion of secreted protein is reabsorbed (up to 0.79; Souffrant et al., 1993) and does not reach the terminal ileum, and losses also occur during synthesis of endogenous protein. Therefore, in this study an alternative approach was used, which allows to quantify the total amount of losses associated with dietary fibre: The reduction of N retention caused by fibre supplementation to a thr limiting diet is taken as a sensitive indicator for increased fibre associated thr losses. Thr is considered in this study, because its concentration in endogenous protein is high (Jansman et al., 2002) and, therefore, a possible reduction of the amount of thr available above maintenance will highly affect N retention.

2.3 MATERIAL AND METHODS

Two experiments were conducted, studying in Exp. 1 the effects of two levels of wheat bran fibre (WBF) and of thr on N retention, and in Exp. 2, the respective effects of rapeseed fibre (RSF), cassava leaf fibre (CLF), and cassava root peel fibre (CRF).

2.3.1 ANIMALS AND HOUSING

In Exp. 1 as well as in Exp. 2, twelve castrated male pigs (Large White X German Landrace) were used with an initial body weight (BW) of 30 ± 3 kg and 26 ± 2 kg, respectively. The pigs were obtained from the Kiel University Research Station. Pigs were housed individually in metabolic crates (0.70 m x 1.75 m), which allowed separate collection of urine and faeces, equipped with a drinking nipple which allowed free access to water. Room temperature was 23 °C where animals were kept and the stable was lighted from 07:30 to 17:30 h.

2.3.2 EXPERIMENTAL PROCEDURE

In Exp. 1, the metabolism trial covered two successive N balance periods. The adaptation of the animals to the environment and the basal diet lasted 35 days, and to the experimental diets 7 days, followed by 7 days for collection of faeces and urine. Three of the 12 pigs were randomly assigned to one of the four experimental diets (1.35 kg basal diet (B (1.35)), 1.15 kg basal diet (B (1.15)), 1.35 kg basal diet + 150 g WBF (B+WBF (150)), and 1.35 kg basal diet + 300 g WBF (B+WBF (300)). For the second balance period, the pigs were assigned to the four diets such that none of the pigs received the same diet twice. Therefore, the study was conducted as a 2 x 4 Youden square design (incomplete Latin square) with a completely randomised block design in which a total of four treatments were tested in two periods and six observations per treatment were obtained. Faeces and urine were collected quantitatively twice daily after feeding and stored at -20°C. To avoid ammonia losses, urine was collected in bottles containing 20 ml H_2SO_4 (20%, v/v) to keep pH values below 2. After the collection periods, total faeces and urine were thawed and homogenised and samples were taken for subsequent analyses. BW of the pigs was measured at the beginning and the end of each collection period.

In Exp. 2, the metabolism trial covered three successive N balance periods. Consequently, nine observations per treatment were obtained. The study was conducted as a 3 x 4 Youden

square design with a completely randomised block design in which a total of four treatments were tested in three periods. The four experimental diets were 1.35 kg basal diet (B (1.35)), basal diet + 150 g RSF (B+RSF), basal diet + 150 g CLF (B+CLF), basal diet + 150 g CRF (B+CRF). The experimental procedures were identical to Exp. 1 except for the length of the adaptation period to the environment and the basal diet which lasted 14 days.

2.3.3 DIETS AND FEEDING

The basal diets consisted of wheat, wheat gluten, wheat bran, extracted soybean meal, soybean oil, and a mineral premix supplemented with lysine-HCl, tryptophan, valine, and isoleucine of which 1.35 kg/d were ingested in all experimental periods (Table 2.1). In Exp. 1, group B (1.15) received a reduced amount of 1.15 kg/d of the basal diet and additionally 150 g/d of corn starch to determine the effect of thr on N retention at similar energy intake and unchanged amino acid pattern. Thr was the first-limiting amino acid in all the experimental diets. To ensure a constantly high energy intake of 1.25 MJ ME/kg $BW^{0.75}$ with increasing BW without any change in protein intake all groups received in addition 240 g/d of corn starch in the second period (Table 2.5a). By this it was ensured that energy intake did not limit N retention (according to recommendations of NRC, 1998 and GfE, 2008). The experimental groups B+WBF (150) and B+WBF (300) received additionally 150 and 300 g/d of the wheat bran fibre, respectively. Composition of the basal diet and the fibre sources are given in Table 1 and 2. Basal diets, corn starch, and fibre sources were mixed and fed in wet-mash form (water : feed = 1 : 1) in two equal meals at 07:30 and 16:30 h.

In Exp. 2, all four experimental groups received the basal diet with 1.35 kg/d and three of those additional 150 g/d fibre from rape seed, cassava leaves, or cassava root peels, respectively. To ensure a constant energy intake of 1.25 MJ ME/kg $BW^{0.75}$ with increasing BW without changing protein intake additional 240 g/d corn starch were provided in the second and 400 g/d in the third period (Table 2.5b). Compositions of the basal diets in both experiments were similar (Table 2.1). The cassava feedstuffs were imported from Nigeria. The other feed ingredients were obtained from commercial sources in Germany. The original feedstuffs could not be used to determine the effect of the respective fibre, because they contained considerable amounts of other nutrients affecting endogenous losses or N retention as well. Therefore, fibre concentrations were increased by removing other components according to the following procedure: The feedstuffs were placed in stuff bags (15 x 25 cm^2), where only a small proportion of the material was washed out due to particle size. Starch of wheat bran and cassava peels was solubilised by boiling 1.5 kg in a mixture of 75 ml alpha-amylase (Termamyl type 120 L, Novo Industrials, Bagsvard, Denmark) and 15 l

water for one hour. The remaining substrate was rinsed with water and then boiled in 15 l neutral detergent solution (according to Van Soest, 1991) for 70 minutes, rinsed again, and dried for 48 h at 70°C. Cassava leaves and rapeseed meal were incubated in a 1% pepsin solution (0.7 FIP-U/mg) for 16 hours at 39°C and mixed three times during this period to remove protein. The substrate was rinsed with water and 1.5 kg of the remaining substrate boiled in 15 l neutral detergent solution for 70 minutes, rinsed again, and dried for 48 h at 70°C.

Table 2.1

The chemical composition of the basal diets and the fibre sources (g/kg DM)

	B Exp. 1	B Exp. 2	WBF	RSF	CLF	CRF
Dry matter (DM), g/kg feed	878	889	918	903	930	918
Components, g/kg DM						
Crude ash	55	62	28	46	79	84
Crude protein	198	195	138	213	240	60
Crude fat	30	30	78	46	42	15
Crude fibre	30	31	193	336	223	374
NDForg	162	162	856	583	653	721
ADForg	41	49	241	497	472	617
ADL	15	15	84	228	269	342
Starch	489	475	29	7	11	140
Sugar	24	28	2	6	8	9

B: basal diet, WBF: wheat bran fibre, RSF: rapeseed fibre, CLF: cassava leaf fibre, CRF: cassava root peel fibre, NDF: neutral detergent fibre, ADF: acid detergent fibre, ADL: acid detergent lignin
Basal diet consisted of (g/kg): wheat 741.5; soybean meal 70; wheat bran 50; wheat gluten 60; mineral-vitamin-premix 40; soybean oil 30; lysine-HCl 5.8; valine 1.5; isoleucine 0.7; tryptophan 0.5; mineral-vitamin-premix contributed to the basal diet (per kg DM): Ca 8.0 g; P 2.0 g; Na 2.0 g; Mg 0.4 g; Fe 160 mg; Cu 40 mg; Mn 80 mg; Zn 160 mg; I 2.0 mg; Se 0.6 mg; Co 0.8 mg; vitamin A 7410 IU; vitamin D 890 IU; vitamin E 59 mg; vitamin K 1.5 mg; vitamin B1 0.7 mg; vitamin B2 3 mg; vitamin B6 1.5 mg; vitamin B12 0.02 mg; niacin 7.4 mg; pantothenic acid 3 mg

Table 2.2

Amino acid contents of the basal diets and the fibre sources (g/kg DM)

	B Exp. 1	B Exp. 2	WBF	RSF	CLF	CRF
Essential amino acids						
Lysine	9.7	11.0	6.6	14.5	13.7	3.3
Methionine	2.9	2.9	2.3	3.9	4.5	2.6
Cystine*	3.8	3.8	2.6	4.5	2.5	0.9
Threonine	5.8	5.7	5.4	11.7	11.1	0.7
Arginine*	9.3	9.4	11.1	12.5	14.2	2.8
Isoleucine	7.6	7.7	4.7	9.9	12.0	2.5
Leucine	13.4	13.4	9.7	15.3	12.2	4.4
Valine	9.7	9.8	7.5	13.3	12.1	3.3
Histidine	4.5	4.5	4.2	5.6	5.3	1.4
Phenylalanine	9.3	9.4	6.1	9.5	14.2	2.6
Total EAA	76.0	77.6	60.2	100.7	101.8	24.5
Non-essential amino acids						
Tyrosine	6.0	6.0	4.2	9.1	11.1	2.2
Glycine	7.6	7.6	8.5	11.2	14.1	2.7
Serine	9.1	9.1	6.6	10.7	11.2	3.2
Proline	17.6	17.8	7.6	15.6	13.3	3.2
Alanine	6.7	6.7	8.0	9.7	14.1	2.9
Aspartic acid	11.0	11.0	10.9	15.5	22.8	5.0
Glutamic acid	56.1	56.0	21.9	27.8	25.5	6.2
Total NEAA	114.1	114.2	67.7	99.6	112.2	25.4

B: basal diet, WBF: wheat bran fibre, RSF: rapeseed fibre, CLF: cassava leaf fibre, CRF: cassava root peel fibre, DM: dry matter, EAA: essential amino acids, NEAA: non-essential amino acids

* conditionally non-essential

2.3.4 ANALYTICAL PROCEDURES

N concentration in faeces and urine was determined in the original substance according to the Kjeldahl procedure. Dry matter (DM) of faeces was determined by freeze-drying and subsequent oven-drying at 105°C overnight. Feeds and freeze-dried faecal samples were ground in a mill (ZM 100, Retsch, Haan, Germany) with a 1 mm screen and, for starch analysis, with a 0.2 mm screen. The Weende method (VDLUFA, 1976) was utilized to determine crude fibre, crude fat, and crude ash. Amino acid analyses were carried out twice according to the methods of the Ajinomoto Eurolysine (Directive 1998/64/EC) and according to Degussa AG (Directive 1998/64/EC). The mean values were taken for further calculations. Gross energy contents of feeds and faeces were determined after complete incineration in an adiabatic bomb calorimeter, whereas the energy content of urine was assumed to be 40 kJ/g N (Hoffmann et al., 1971). The ADF and NDF (with and without residual ash) were analysed according to Van Soest et al. (1991) without the use of decalin. Starch content was determined by enzyme hydrolysis of starch to glucose, employing the heat-stable alpha-amylase Termamyl type 120 L (Brandt et al., 1987). The in vitro digestibility

of dry matter and protein of the feedstuffs were determined according to the method of Boisen (1997). In vitro digestibility was assumed to be identical with standardized precaecal protein digestibility and not different from standardized pc. thr digestibility. For further characterisation of the fibre source, water-holding capacity was determined by the method of Thibault et al. (1992). The particle size distribution of the feedstuffs was determined by dry sieving for 12 min, using sieves (20 cm i.d.) with square apertures of 0.063, 0.125, 0.25, 0.5, 1, 2 and 4 mm on a side, with an electromagnetic sieve shaker (Vibrotonic type VE-1, Retsch, Haan, Germany). The sieve shaker was operating with an amplitude of 2 mm and the vibrations were interrupted every 20 sec for approximately 2 sec. The water-holding capacity and the particle size distribution of the basal diets and the fibre sources are given in Table 2.3.

Table 2.3
Particle size distribution and water-holding capacity of the basal diets and the fibre sources

	B Exp. 1	B Exp. 2	WBF	RSF	CLF	CRF
			Proportion*			
Particle size (mm)						
>4	0.00	0.00	0.02	0.08	0.06	0.04
>2-4	0.04	0.12	0.18	0.18	0.15	0.28
>1-2	0.27	0.32	0.56	0.24	0.19	0.44
>0.5-1	0.31	0.26	0.23	0.35	0.28	0.21
≤ 0.5	0.38	0.31	0.01	0.15	0.32	0.03
Water-holding capacity** (g water/g fibre source)			8.97	8.43	7.14	6.12

B: basal diet, WBF: wheat bran fibre, RSF: rapeseed fibre, CLF: cassava leaf fibre, CRF: cassava root fibre
* expressed as weight proportion of total
** every value consistent of two observations. The mean variance was 0.78, 0.24, 0.66, and 0.59 for WBF, RSF, CLF, and CRF.

2.3.5 STATISTICAL ANALYSES AND CALCULATIONS

Statistical analyses were carried out with the mixed model procedure (Proc Mixed) of SAS (1996) using the covariance structure heterogenous compound symmetry. The model used is: $Yijk = \mu + Di + Pj + Ak + eijk$, where $Yijk$ is observed response, μ the overall mean, Di the effects of diet i, Pj effect of period j, Ak effect of animal k and $eijk$ is the residual error. Diet and period are fixed effects and animal and residual errors were random components. A pairwise comparison between Least Squares Means was conducted with the Tukey-Kramer-Test. N retentions were corrected for pcd. thr intake originating from the fibre sources according to the thr effect on N retention which was determined by the difference between group B(1.35) and B(1.15) in Exp. 1.

2.4 RESULTS

The experiments were carried out without any problem regarding illness. All diets offered to the animals were completely ingested at every meal. In the first experiment the data of one animal had to be excluded, because the amount of urine was twenty times as high as the amount of the remaining animals. The digestibility of the basal diets and supplemented fibre sources in Exp. 1 and 2 are shown in Table 2.4.

Table 2.4a+b

Digestibility (%) of basal diets and supplemented fibre sources in Exp. 1 and 2

a) Exp. 1

Diet	B (1.35)	B (1.15)	SEM	WBF (150)	WBF (300)	SEM	P
Parameters							
DM (%)	89.5[a]	90.4[a]	0.2	40.6[b]	37.4[b]	1.4	<0.001
OM (%)	87.2[a]	88.6[a]	0.3	41.3[b]	39.6[b]	2.3	<0.001
CP (%)	89.2[a]	88.3[a]	0.8	35.9[b]	37.0[b]	3.2	<0.001
CF (%)	45.0[a]	36.5[b]	1.6	15.9[b]	25.5[b]	3.3	0.038
NDF (%)	69.5[a]	68.2[a]	0.7	45.0[b]	47.3[b]	1.4	<0.001
ADF (%)	35.6[a]	32.3[a]	1.4	12.6[b]	15.9[b]	2.5	<0.001
ADL (%)	35.6[a]	40.6[a]	3.5	8.0[b]	15.7[b]	3.1	0.003
GE (%)	86.4[a]	85.8[a]	0.2	40.9[b]	36.8[b]	1.9	<0.001

b) Exp. 2

Diet	B (1.35)	SEM	RSF	CLF	CRF	SEM	P
Parameters							
DM (%)	88.0	0.1	34.5[a]	21.7[b]	13.5[b]	3.1	0.002
OM (%)	87.9	0.1	36.0[a]	22.7[b]	22.4[b]	3.1	0.016
CP (%)	89.0	0.4	28.3[a]	11.8[a]	-82.0[b]	9.1	<0.001
CF (%)	35.8	0.8	20.1[b]	29.5[a]	17.3[b]	2.9	0.041
NDF (%)	64.9	0.3	50.3[a]	39.7[b]	19.4[c]	3.1	<0.001
ADF (%)	40.7	0.7	17.6[a]	13.0[ab]	1.3[b]	3.8	0.035
ADL (%)	42.1	1.7	-11.4[b]	-3.4[b]	12.7[a]	4.3	0.008
GE (%)	86.2	0.2	34.3[a]	22.6[b]	18.5[b]	7.0	0.011

B(1.35): 1.35 kg/d basal diet, B(1.15): 1.15 kg/d basal diet, WBF(150): 150 g/d wheat bran fibre, WBF(300): 300 g/d wheat bran fibre, RSF: rapeseed fibre, CLF: cassava leave fibre, CRF: cassava root peel fibre, DM: dry matter, OM: organic matter, N: nitrogen, CF: crude fibre, NDF: neutral detergent fibre, ADF: acid detergent fibre, ADL: acid detergent lignin, GE: gross energy

The digestibility did not differ between the basal diets. The fibre sources showed lower digestibilities in all components compared with the basal diets. The level of WBF intake did not affect its digestibility, while the respective digestibility of OM, CP, and the different fibre fractions considerably differed between RSF, CLF, and CRF. The negative value for crude protein digestibility of CRF could have been caused by the low protein concentration and by

microbial activity in the hind gut. Feed intake and N balance in Exp. 1 and 2 are shown in Table 2.5.

Table 2.5a+b

Feed intake and N balance in Exp. 1 and 2
a) Exp. 1

Diet / Parameters	B (1.35)	B (1.15)	B + WBF (150)	B + WBF (300)	SEM	P
Feed intake (DM g/d)						
Basal diet	1194	1017	1194	1194		
Corn starch[1**]	209	341	209	209		
Corn starch[2***]	350	480	350	350		
Fibre source	-	-	138	276		
total thr intake	6.9	5.9	7.6	8.4		
pcd. thr intake	6.4	5.4	6.8	7.3		
N balance (g/day)						
N intake	37.9[c]	32.3[d]	40.9[b]	44.0[a]	0.005	<0.001
Faecal N	4.1[c]	3.7[c]	6.1[b]	7.9[a]	0.25	<0.001
Urinary N	15.4[b]	15.2[b]	16.5[ab]	17.7[a]	0.56	0.049
Total N excretion	19.5[b]	18.9[b]	22.5[a]	25.6[a]	0.62	<0.001
N retention	18.3[a]	13.3[b]	18.4[a]	18.4[a]	0.61	0.002
N balance (g/g N intake)						
Faecal N	0.108[c]	0.117[c]	0.149[b]	0.179[a]	0.006	<0.001
Urinary N	0.410	0.466	0.400	0.405	0.015	0.066
Total N excretion	0.518	0.583	0.549	0.579	0.017	0.104
N retention	0.482	0.417	0.451	0.415	0.017	0.104

b) Exp. 2

Diet	B (1.35)	B + RSF	B + CLF	B +CRF	SEM	P
Parameters						
Feed intake (DM g/d)						
Basal diet	1199	1199	1199	1199		
Corn starch[1*]	-	-	-	-		
Corn starch[2**]	209	209	209	209		
Corn starch[3***]	350	350	350	350		
Fibre source	-	134	138	137		
total thr intake	6.9	8.4	8.4	7.0		
pcd. thr intake	6.4	7.1	6.7	6.4		
N balance (g/day)						
N intake	37.4[d]	42.0[b]	42.7[a]	38.7[c]	0.01	<0.001
Faecal N	4.1[c]	7.3[b]	8.8[a]	6.6[b]	0.16	<0.001
Urinary N	15.1	14.9	14.3	14.2	0.29	0.143
Total N excretion	19.2[d]	22.2[b]	23.2[a]	20.9[c]	0.28	<0.001
N retention	18.3[b]	19.8[a]	19.5[a]	17.9[b]	0.29	<0.001
N balance (g/g N intake)						
Faecal N	0.109[c]	0.174[b]	0.207[a]	0.172[b]	0.004	<0.001
Urinary N	0.387	0.351	0.338	0.369	0.013	0.092
Total N excretion	0.496	0.525	0.545	0.541	0.014	0.088
N retention	0.488	0.471	0.460	0.461	0.008	0.061

B(1.35): 1.35 kg/d basal diet, B(1.15): 1.15 kg/d basal diet, WBF(150): 150 g/d wheat bran fibre, WBF(300): 300 g/d wheat bran fibre, RSF: rapeseed fibre, CLF: cassava leave fibre, CRF: cassava root peel fibre, DM: dry matter, pcd.: praecaecal digestible, thr: threonine, N: nitrogen
1: Intake during the first balance period.
2: Intake during the second balance period.
3: Intake during the third balance period.
* mean BW: 40.6 kg; ** mean BW: 55.1 kg; ***mean BW: 69.1 kg

Faecal N excretion expressed quantitatively as well as in fractions of N intake increased when the fibre sources were added to the basal diets ($p < 0.001$). Urinary N increased when feeding WBF (300) ($p = 0.049$). Total N excretion increased by adding the fibre sources to the basal diets ($p < 0.001$). N retention in B (1.15) was lower ($p = 0.002$) than in the basal diet showing the extent of the effect of thr intake. N retentions in B+RSF and B+CLF were higher ($p < 0.001$) than in the basal diet, whereas N retentions in B+CRF and the two B+WBF did not differ from the basal diets. N retentions expressed as fractions of N intake were lower in B+WBF (300), B+CLF, and B+CRF, however in B+WBF (150) and B+RSF no difference to the basal diets was observed. In both experiments faecal N excretion decreased with period ($p = 0.008$) and, therefore, N digestibility was affected by period ($P < 0.001$), however urinary N excretion and N retention were not different between periods. The animal did not have any influence on N-balance parameters. In vitro and in vivo N digestibility and corrected N retentions are shown in Table 2.6.

Table 2.6a+b

In vitro and in vivo N-digestibility and corrected N retentions in Exp. 1 and 2
a) Exp. 1

Diet / Parameters	B (1.35)	B (1.15)	B + WBF (150)	B + WBF (300)	SEM	P
Dig. of N (%) in vivo	89.2[a]	88.3[a]	85.1[b]	82.0[c]	0.59	<0.001
Dig. of N (%) in vitro	92.1[a]	92.1[a]	89.0[b]	86.5[c]	0.001	<0.001
N retention (g/d)	18.3[a]	13.3[b]	18.4[a]	18.4[a]	0.61	0.002
pcd. thr intake (g/d)	6.4	5.4	6.8	7.3	0.013	<0.001
pcd. thr intake (g/d)*	0	0	0.5	0.9	0.001	<0.001
N retention corr. (1)**	18.3[a]	13.3[c]	15.9[b]	13.5[c]	0.61	0.007
N retention corr. (2)**	18.3[a]	13.3[c]	17.2[ab]	15.9[b]	0.62	0.007

b) Exp. 2

Diet / Parameters	B (1.35)	B + RSF	B + CLF	B +CRF	SEM	P
Dig. of N (%) in vivo	89.0[a]	82.6[b]	79.2[c]	82.7[b]	0.61	<0.001
Dig. of N (%) in vitro	92.2[a]	87.6[b]	85.3[c]	85.0[d]	0.001	<0.001
N retention (g/d)	18.3[b]	19.8[a]	19.5[a]	17.9[b]	0.29	<0.001
pcd. thr intake (g/d)	6.4	7.1	6.7	6.4	0.007	<0.001
pcd. thr intake (g/d)*	0	0.7	0.4	0.1	0.002	<0.001
N retention corr. (1)**	18.3[a]	16.0[b]	17.5[a]	17.5[a]	0.29	<0.001
N retention corr. (2)**	18.3[a]	17.9	18.5	17.7	0.29	0.204

B(1.35): 1.35 kg/d basal diet, B(1.15): 1.15 kg/d basal diet, WBF(150): 150 g/d wheat bran fibre, WBF(300): 300 g/d wheat bran fibre, RSF: rapeseed fibre, CLF: cassava leave fibre, CRF: cassava root peel fibre, dig.: digestibility, N: nitrogen, pcd.: precaecal digestible, thr: threonine, corr.: corrected
*pcd. thr intake from the fibre source.
**corrected for additional pcd. thr intake from the fibre source according to the thr effect determined in Exp. 1 and according to an assumed value of 2.6 g RN/g (Exp. 2, for details see chapter discussion).

N retentions were corrected for pc. digestible thr intake originating from the fibre sources according to the thr effect on N retention determined in Exp. 1 (B(1.35) and B(1.15)), where 1 g pcd. thr intake resulted in 5.3 g N retention (correction 1). In vitro and in vivo N digestibility, intake of pcd. thr, as well as corrected N retentions are shown in Table 2.6 WBF and RSF reduced corrected N retentions ($P < 0.01$), whereas CLF and CRF did not show a significant negative effect. The higher amount of WBF (300) resulted in a further decrease of N retention when compared to WBF (150) ($P = 0.007$).

2.5 DISCUSSION

As expected, DM, fibre, and N digestibilities were reduced in diets supplemented with the different fibre sources, which confirms the observations reported in the literature (Chabeauti et al., 1991; Noblet et al., 1994). In the present study in vivo N digestibility was 0.89 and 0.82 and in vitro N digestibility 0.92 and 0.86 in B and B+WBF (220 g WBF/kg diet),

respectively. Using similar diets Chabeauti et al. (1991) reported in vivo N digestibilities of 0.92 and 0.87 in their basal and wheat bran supplemented (220 g wheat bran/kg) diets. Fibre in the diet may reduce apparent protein and AA digestibility due to reduced protein and amino acid absorption and/or increased endogenous secretion (Schulze et. al., 1994) and higher microbial growth due to enhanced hind gut fermentation.

NDF digestibility of the fibre sources used in this study is only in part in agreement with values given in feed tables (DLG, 1984 and CVB, 2000, where digestible fibre is defined as sum of digestible crude fibre and N free extracts minus starch and sugar). NDF digestibility was 0.45, 0.48 and, 0.20 for WBF, RSF, and CRF. Fibre digestibilities according the feed table of DLG (1984) are 0.45, 0.54, and 0.45 for wheat bran, rapeseed, and cassava chips, and according to the fed table of CVB (2000) 0.30, 0.46, and 0.43, respectively. However, fibre digestibility of cassava root chips as given in feed tables might be higher than of the root peel fibre used in the present study. NDF digestibility for cassava leaves was 0.42, while a value of 0.52 was reported by Phuc et al. (2000).

Generally, faecal N excretion increased ($P < 0.001$) and urinary N excretion tended to decrease by adding fibre to the basal diet. This is in agreement with results of e.g. Canh et al. (1997), who ascertained that pigs fed fibre-rich by-product diets excreted less N via urine and more N via faeces due to a higher amount of bacterial matter than pigs fed grain-based diets.

N retention remained unchanged by adding WBF and CRF and even significantly increased by adding RSF and CLF. The negative effects of fibre on N retention did not occur, since additional thr intake originating from the fibre sources compensated for those effects. Due to this reason, N retentions were corrected for pcd. thr intake from the fibre sources. In vitro digestibility of protein determined according to the method of Boisen (1997) was used as an estimate for pcd. protein. To proof validity of using in vitro results for in vivo pc. digestibility, mean standardized pc. digestibility of protein of the basal diets was calculated according to the feed table of GfE (2008) which resulted in 0.89, which agrees well with the in vitro value of 0.92. In vitro digestibility of protein of the fibre sources were used as an estimate of their pc. digestibility. Furthermore, it was assumed that pc. digestibility of protein does not differ from pc. digestibility of thr. This further assumption seems to be valid as well, since standardized pc. digestibility of protein is 0.90, 0.72, and 0.82 and of thr 0.91, 0.72, and 0.86 for wheat, wheat bran, and soybean meal, respectively, representing the main components of the basal diet (values from GfE, 2008).

Due to the fact that the thr effect as determined in the present study was considerable higher (5.3 g N retention/g thr) than would be expected from the efficiency of utilisation of ideal protein (2.6 g N retention/g pcd. thr; GfE, 2008), N retentions were corrected using this lower value (correction 2) as well. According to correction 2, N retention was reduced in B+WBF

(300) (P = 0.007), whereas RSF now did not show any negative effect. The effects of CLF and CRF on N retention did not differ from those obtained when applying correction 1 (Table 2.6).

Fibre associated thr losses can be derived by equating a reduction of 5.3 g corrected N retention with a reduction of pcd. thr available for protein retention by 1 g. Then these losses amount to 3.2, 3.3, 3.2, 1.2, and 1.1 g/kg WBF (150), WBF (300), RSF, CLF, and CRF, and 3.8, 3.9, 5.5, 1.8, and 1.5 g/kg NDF, respectively. Thr losses were not affected by level of WBF, were higher in RSF and lower in CLF and CRF. When using the theoretical value (2.6 g N retention/g pcd. thr) thr losses were 3.1, 3.2, 1.1, and 1.7 g/kg WBF (150), WBF (300), RSF, and CRF, and 3.6, 3.8, 1.8, and 2.3 g/kg NDF, respectively, whereby CLF did not seem to affect thr losses. Using the value of 2.6 instead of 5.3 g N/g pcd. thr resulted in similar effects for WBF (150), WBF (300), and CRF, while the negative effects of RSF and CLF disappeared.

Independent of the mode of calculation, the fibre sources clearly showed negative effects on N retention, which indicates increased endogenous thr losses or reduced thr absorption. WBF and RSF caused larger effects than CLF and CRF, but reasons for these differences could not be conclusively attributed. Particle size and water-holding capacity are seen by Dierick et al. (1989) and Souffrant (2001) as factors affecting endogenous losses, however these values are not related to the findings in the present study.

Fibre associated thr losses as determined in the present study cannot be compared directly to values of endogenous losses reported in the literature, since the latter represent the amounts of thr of endogenous origin at the end of the ileum, whereas our approach includes all losses associated with fibre including those during synthesis of endogenous protein. Nevertheless, all values are compiled and presented in Table 2.7.

Table 2.7

Endogenous precaecal threonine (thr) losses in different diets reported in the literature and fibre source associated thr losses determined in the present study (g/kg DM)

Authors	Experimental approach	Feed to which losses are related	NDF g/kg feed	Endogenous precaecal thr losses
De Lange et al. (1989)	Protein free	basal diet (cornstarch, cellulose, sucrose)	30	0.65
De Lange et al. (1989)	Protein free	basal diet + cellulose	100	0.72
Jansman et al. (2002)	*	N-free diet	< 80	0.51
Jansman et al. (2002)	*	casein/wheat gluten diet	< 80	0.72
Dilger et al. (2004)	Casein as protein source	low-protein, casein-based diet	50	0.73
Steendam et al. (2004)	Casein as protein source, ^{15}N-IDT	basal diet (cornstarch, casein)	0	0.62
Steendam et al. (2004)	Casein as protein source, ^{15}N-IDT	basal diet + quebracho extract	not reported	1.70
				Fibre source associated thr losses
Present study	Effect on N retention	cassava root fibre	721	1.11
	in thr limited diets	cassava leaf fibre	653	1.16
		wheat bran fibre (150)	856	3.23
		wheat bran fibre (300)	856	3.30
		rapeseed fibre	583	3.23

N: nitrogen, DM: dry matter, ^{15}N-IDT: ^{15}N-isotope dilution technique
* mean of different experimental approaches considered in this review.

Diets low in fibre concentration show a range in endogenous losses between 0.51 and 0.73 g thr/kg DM and it is obvious than losses increase with increasing NDF content and depend on fibre source. Therefore, it is evident that independent of the methods applied, the concentration and source of fibre in the diet have to be taken into account in addition to the amount of feed ingested when endogenous losses have to be estimated. Furthermore, it has to be concluded that high fibre diets increase the thr requirement of the pigs. Usually the AA maintenance requirements are related to BW (NRC, 1998; GfE, 2008), however these figures are derived from studies using diets with very low NDF contents and for this reason underestimate endogenous losses and consequently the thr requirement. The estimate of Fuller et al. (1989) for thr maintenance requirement of 53 mg/kg $BW^{0.75}$ was derived in diets containing 50 - 60 g fibre/kg, the value of Heger et al. (2002) in diets with 55 g fibre/kg, and the mean value for the basal ileal endogenous thr flow given by Jansman et al. (2002) was determined for diets containing less than < 80 g NDF/kg (Table 2.7). Therefore, it has to be discussed whether those values are appropriate figures for pigs fed commercial diets with at least a two-fold higher fibre concentration. Assuming that 50 mg pcd. thr/kg $BW^{0.75}$ (GfE, 2008) is an appropriate estimate of the maintenance requirement in diets with 60 g NDF/kg, a diet with 180 g NDF/kg would increase (according to our estimate of 3.8 pcd. thr/kg NDF)

the maintenance requirement of a pig weighing 80 kg and ingesting 2.75 kg/d of feed from 1.3 to 2.4 g pcd. thr/d.

2.6 CONCLUSIONS

It can be concluded from the results of this study that fibre of specific feeds decreases N retention in thr limiting diets due to increased losses of thr. Therefore, fibre source and concentration in the diet should be considered as a significant factor affecting thr maintenance requirement.

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3 OPTIMUM THREONINE : LYSINE RATIO IN DIETS FOR GROWING PIGS: AN EVALUATION OF LITERATURE DATA

3 OPTIMUM THREONINE : LYSINE RATIO IN DIETS FOR GROWING PIGS: AN EVALUATION OF LITERATURE DATA

3.1 ABSTRACT

Threonine requirement is usually expressed relative to that of lysine. However, reports in the literature on the optimum threonine : lysine ratio ($T:L_{opt}$) for growing pigs considerably vary. This paper summarizes all available data on $T:L_{opt}$ and tries to identify possible factors responsible for its variation. 65 experiments performed to determine the $T:L_{opt}$ in diets of growing pigs were found in the literature published between 1977 and 2007. However only those studies could be considered where threonine was the first-limiting and lysine the second-limiting nutrient. The optimum threonine supply is defined as the T:L ratio at the beginning of the plateau phase of the dose-response relationship of threonine. The experimental response parameters in most of the studies were feed intake, body weight gain, feed:gain ratio, and in some studies N balance or plasma urea concentration. Within each experiment the highest value for $T:L_{opt}$ received for the different parameters was taken for statistical analysis. In 15 experiments $T:L_{opt}$ could not be defined, because $T:L_{opt}$ was below the lowest T:L ratio or above the highest T:L ratio tested. The overall mean for $T:L_{opt}$ is 0.61 (SD = 0.07; n = 40) and on a precaecal digestible amino acid basis 0.57 (SD = 0.10; n = 40). $T:L_{opt}$ seems to be unaffected by body weight, body weight gain or crude protein and lysine concentration in the diet, whereas $T:L_{opt}$ increased significantly with NDF concentration in the diet. The specific effect of fibre on the threonine requirement is in accordance with recent studies. The mean of 0.61 for $T:L_{opt}$ cannot be taken as a general recommendation due to the following reasons: (1) Ignoring the relatively large variation (SD = 0.07) would obviously lead to a high risk in undersupply with threonine. (2) Because of the low fibre contents in most of the experimental diets (90 g NDF/kg DM), threonine requirement can be expected to be considerably higher in commercial diets. According to the relationship between $T:L_{opt}$ and NDF content derived from these data $T:L_{opt}$ increases from 0.62 at 90 g to 0.68 at 125 g NDF/kg diet dry matter. It is concluded that optimum threonine supply to pigs should not be defined without considering the fibre concentration in their diets.

KEYWORDS: Pig, Threonine, Lysine

3.2 INTRODUCTION

The knowledge of the critical ratios of essential AA is a prerequisite for diet formulations. Its importance increases in protein-reduced diets and when free AA are supplemented. Threonine is the second-limiting AA in wheat and barley and the third-limiting AA in corn (Lewis and Peo, 1986). Threonine plays a specific role for the protein metabolism of the gastrointestinal tract, because especially glycoproteins of the mucus beside salvia and gastric secretion are high in threonine (Kluge et al., 2002). Therefore, threonine requirement may specifically increase with increasing endogenous secretion to a larger extent than in other AA. Due to this reason increasing proportions of fibre rich feeds in diets may have a specific effect on the threonine requirement and, therefore, may modify the critical ratio to other AA, because fibre is discussed to act as an anti-nutritional factor by reducing apparent pc. protein and AA digestibility due to reduced absorption and/or increased endogenous secretion (Schulze et al., 1994). The requirement of threonine for maintenance relative to lysine is considerably higher than for growth (Kluge et al., 2002) and the experiments of Wang et al. (1989) showed that the T:L ratio of the requirement for maintenance is 1.47:1 and for growth only 0.53:1. As a consequence dietary optimum threonine : lysine ratio ($T:L_{opt}$) for pigs increases with body weight (BW) (Tuitoek et al., 1997; Pedersen et al., 2003). According to Berende et al. (1983) further factors may influence this ratio as well: genetic origin, gender, dietary factors (protein, energy), and environmental conditions (e.g. state of hygiene and temperature). Therefore, values for the dietary $T:L_{opt}$ in growing pigs reported in the literature differ to a great extent, most likely because they were obtained under different experimental conditions. The aim of this study is (i) to summarize all data on $T:L_{opt}$ available in the literature and (ii) to try to identify factors affecting this ratio.

3.3 MATERIAL AND METHODS

Sixty-five experiments were found in the literature, published between 1977 and 2007, which were performed to determine $T:L_{opt}$ in diets of growing pigs. However, for statistical analysis only those experiments could be included in which threonine was the first-limiting and lysine the second-limiting AA and no other factors limited growth. Experiments where the content of lysine in the diets was not reported or could not be calculated due to missing information on feedstuffs used, were excluded because T:L ratio could not be defined. The experimental parameters recorded were in nearly all studies feed intake, BW gain and feed:gain ratio, in few studies N balance and plasma urea concentrations, and in two studies immune function. In Table 1, the authors of the respective studies, BW range of animals; lysine, crude protein, and NDF concentration of the diets; feed allowance, threonine

concentrations as well as T:L ratios tested, and response criteria are compiled. The $T:L_{opt}$ was derived from the published data for each experiment according to the following directives:

- $T:L_{opt}$ is the T:L ratio at the beginning of the plateau phase of the dose-response relationship. The ratio below ($T:L_{opt-1}$) was not considered as optimum, even if $T:L_{opt}$ tended only to be higher than $T:L_{opt-1}$.
- If animals responded to the different T:L ratios, but did not reach the plateau phase, $T:L_{opt}$ could not be defined, since it is above the highest T:L ratio tested.
- If animals did not respond to different T:L ratios, $T:L_{opt}$ could not be defined as well, since it was below the lowest T:L ratio tested.
- Within an experiment that response criterion was selected which lead to highest value for $T:L_{opt}$.

If the contents of apparent pcd. AA, total threonine and lysine, or contents of crude protein were not reported in the publications, these values were calculated on the basis of the feed components used and on their AA composition according to the feed tables of CVB (2000). For all diets the NDF concentration was calculated as well from feed components used and their NDF concentrations according to the feed tables of CVB (2000). BW gain was not reported for experiment 43, 49, and 50, and NDF content of the diet could not be calculated for experiment 10 (Table 3.1). For the experiments of Bikker et al. (2007) the ratio of total AA was assumed to be identical with the ratio of pcd. AA, since concentrations of total AA were not given and could not be recalculated from feed components.

For statistical analysis of the data, descriptive statistics (mean, SD, minimum, 25- and 75-percentiles, maximum, and median) were used, except for the description of the relationships between $T:L_{opt}$ and possible factors affecting $T:L_{opt}$, where the procedure REG of SAS (1996) was used.

Table 3.1
Compilation and description of published studies on optimum threonine : lysine ratios ($T:L_{opt}$) in diets for growing pigs

Authors	Exp. no.[1]	Body weight (kg)	Concentrations of lysine / crude protein / NDF in the diets (g/kg DM)	Feed allowance	Threonine concen-trations tested (g/kg DM)	Threonine to lysine ratios (T:L) tested	Criteria[4]	T:L at beginning of plateau ($T:L_{opt}$)	Difference between $T:L_{opt}$ and T:L below $T:L_{opt}$	Mean of $T:L_{opt}$ and T:L below $T:L_{opt}$	Apparent pcd. T:L at beginning of plateau (pcd. $T:L_{opt}$)	Remarks
Adeola et al. (1994)	1*	10-21	11.2/147/75	Ad libitum	4.5, 5.3, 6.0, 6.7, 7.4	0.40, 0.47, 0.53, 0.60, 0.66	Feed intake* BW gain* Feed:gain*	0.60	0.07	0.57	0.48	[3]
Bartelt et al. (2004)	2	50-80	7.8/142/115	Ad libitum	5.0, 5.3, 5.7, 5.9	0.64, 0.68, 0.73, 0.76	Feed intake BW gain* Feed:gain* N-retention	0.73	0.05	0.71	0.68	
	3	50-80	8.9/157/115	Ad libitum	4.7, 5.0, 5.2, 5.5	0.53, 0.56, 0.58, 0.62	Feed intake BW gain* Feed:gain N-retention	>0.62			0.61	
Berende and Bertram (1983)	4	15-45	12.6/191/80	Restricted (two times / day)	4.5, 5.4, 6.3, 7.2, 8.1	0.36, 0.43, 0.50, 0.57, 0.64	BW gain* Feed:gain*	0.57	0.07	0.54	0.45	[3], castrated males
	5	15-45	12.6/191/80	Restricted (two times / day)	4.5, 5.4, 6.3, 7.2, 8.1	0.36, 0.43, 0.50, 0.57, 0.64	BW gain* Feed:gain*	0.57	0.07	0.54	0.45	[3], females
Bergström et al. (1995)	6*	About 5-15	14.4/169/47	Ad libitum	6.9, 7.4, 8.0, 8.6, 9.2, 9.7	0.48, 0.52, 0.56, 0.60, 0.64, 0.68	Feed intake* BW gain* Feed:gain*	<0.48			<0.50	[2], CP is calculated according to DLG (1984)
	7*	About 5-15	18.5/ 218/41	Ad libitum	9.1, 9.9, 10.6, 11.4, 12.1, 12.9	0.49, 0.54, 0.57, 0.62, 0.65, 0.70	Feed intake* BW gain* Feed:gain*	<0.49			<0.50	[2], CP is calculated according to DLG (1984)
Bergström et al. (1996)	8	12-25	9.8/164/52	Ad libitum	3.8, 4.4, 4.9, 5.5, 6.1	0.39, 0.45, 0.50, 0.56, 0.62	Feed intake* BW gain* Feed:gain*	0.50	0.05	0.48	0.55	[2], CP is calculated according to DLG (1984)
	9	12-25	14.0/214/53	Ad libitum	5.6, 6.4, 7.3, 8.1, 8.9.	0.40, 0.46, 0.52, 0.58, 0.64	Feed intake BW gain* Feed:gain*	0.46	0.06	0.43	0.48	[2], CP is calculated according to DLG (1984)
Bikker et al. (2007)	10	25-45	7.9/not reported	Ad libitum	4.3, 4.7, 5.1, 5.6	0.55, 0.60, 0.65, 0.71	BW gain* Feed:gain*	0.65	0.05	0.63	0.65	only values for pcd. threonine and lysine were reported

	11	45-110	6.7/not reported	Ad libitum	3.7, 4.0, 4.4, 4.8	0.55, 0.60, 0.65, 0.71	BW gain* Feed:gain*	< 0.55			< 0.55	only values for pcd. threonine and lysine were reported
Borg et al. (1987)	12*	Mean initial weight 7.8	11.0/130/93	Ad libitum	5.3, 5.8, 6.3, 6.8, 7.3	0.48, 0.53, 0.57, 0.62, 0.66	Feed intake BW gain Feed:gain*	0.57	0.04	0.55	0.71	[3]
	13*	Mean initial weight 9.7	10.0/120/92	Ad libitum	5.0, 5.7, 6.4, 7.1, 7.8	0.50, 0.57, 0.64, 0.71, 0.78	Feed intake BW gain Feed:gain* Plasma urea*	0.64	0.07	0.61	0.73	[3]
Buraczew-ska et al. (2006)	14	25-50	10.4/180/96	Ad libitum	5.9, 6.5, 7.0, 7.4	0.57, 0.63, 0.67, 0.71	BW gain Feed:gain* N-retention*	0.67	0.04	0.65	0.71	castrated males
	15	25-50	10.5/160/96	Ad libitum	5.6, 6.9	0.53, 0.66	BW gain* Feed:gain* N-retention*	>0.66	0.13	0.60	0.87	castrated males
Cohen and Tanksley (1977)	16	18-40	9.1/180/60	Ad libitum	4.4, 5.1, 5.7, 6.4, 7.1, 7.8	0.48, 0.56, 0.63, 0.70, 0.78, 0.86	Feed intake BW gain* Feed:gain*	0.56	0.08	0.52	0.45	[3]
	17	60-90	6.9/157/58	Ad libitum	3.3, 3.9, 4.6, 5.3, 6.0, 6.7	0.48, 0.56, 0.67, 0.77, 0.87, 0.97	Feed intake BW gain* Feed:gain*	0.56	0.08	0.52	0.43	[3]
Conway et al. (1990)	18	17-50	11.9/164/89	Ad libitum	6.0, 6.5, 7.0, 7.5, 7.9	0.50, 0.55, 0.59, 0.63, 0.66	Feed intake BW gain* Feed:gain*	0.59	0.04	0.57	0.38	[3]
Defa et al. (1999)	19	17.5-30	10.3/179/132	Ad libitum	6.6, 7.6, 8.7, 10.0	0.64, 0.74, 0.84, 0.97	Feed intake BW gain* Feed:gain* Plasma urea* Immunity	0.74	0.10	0.69	0.65	[3]
Edmonds and Baker (1987)	20	Mean initial weight 8	12.9/225/89	Ad libitum	8.4, 9.0, 9.6, 10.7, 12.9	0.65, 0.69, 0.74, 0.87, 1.00	Feed intake* BW gain* Feed:gain*	<0.65			<0.63	[3]
Ettle et al. (2004)	21	35-65	9.4/168/99	Ad libitum	5.3, 5.7, 6.2, 6.5	0.56, 0.61, 0.66, 0.69	Feed intake* BW gain* Feed:gain*	>0.69			>0.69	
	22	35-65	10.8/168/99	Ad libitum	6.1, 6.4, 7.0, 7.6	0.56, 0.59, 0.65, 0.70	Feed intake BW gain* Feed:gain	>0.70			0.65	
	23	65-110	7.3/138/105	Ad libitum	4.2, 4.5, 4.8, 5.4	0.58, 0.62, 0.66, 0.74	Feed intake* BW gain* Feed:gain*	0.62	0.04	0.60	0.60	

	24	65-110	7.9/138/105	Ad libitum	4.2, 4.7, 4.9, 5.3	0.53, 0.59, 0.62, 0.67	Feed intake BW gain* Feed:gain*	0.59	0.06	0.56	0.56	
Goodband et al. (2002)	25	10-20	13.9/185/101	Ad libitum	7.8, 8.0, 8.8, 9.1, 9.6	0.56, 0.58, 0.63, 0.65, 0.69	Feed intake BW gain* Feed:gain*	0.65	0.02	0.64	0.61	
	26	10-20	11.9/170/101	Ad libitum	6.8, 7.0, 7.3, 7.7, 8.3	0.57, 0.59, 0.61, 0.65, 0.70	Feed intake BW gain* Feed:gain*	0.65	0.04	0.63	0.72	
James et al. (2002)	27	12-25	13.6/180/88	Ad libitum	7.2, 7.8, 8.4, 9.0, 9.6	0.53, 0.57, 0.62, 0.66, 0.73	Feed intake* BW gain Feed:gain*	0.62	0.05	0.60	0.60	
	28	12-25	14.8/195/87	Ad libitum	7.9, 8.5, 9.2, 9.9, 10.6	0.53, 0.57, 0.62, 0.67, 0.72	Feed intake BW gain Feed:gain*	0.67	0.05	0.65	0.65	
Kluge et al. (2002)	29	30-50	9.0/164/104	Ad libitum	5.2, 5.9, 6.6, 7.2	0.57, 0.65, 0.74, 0.79	Feed intake BW gain* Feed:gain* N-retention	0.65	0.08	0.61	0.65	
Kovar et al. (1993)	30*	10-20	11.5/139/78	Ad libitum	4.5, 5.0, 5.6, 6.1, 6.7, 7.2	0.39, 0.44, 0.49, 0.54, 0.59, 0.64	Feed intake BW gain* Feed:gain Plasma urea	0.59	0.05	0.57	0.47	[3]
	31*	10-20	13.9/131/78	Ad libitum	4.5, 4.7, 4.9, 5.1, 5.3	0.32, 0.34, 0.35, 0.37, 0.38	Feed intake BW gain* Feed:gain* Plasma urea	>0.38			>0.29	[3]
Leibholz (1988)	32	About 2-10	14.2/222/39	Ad libitum	5.3, 6.3, 7.3, 8.3, 9.3	0.37, 0.44, 0.51, 0.58, 0.65	BW gain* Feed:gain* N-retention*	0.44	0.07	0.41	0.40	[3], castrated males
	33	About 2-10	14.7/221/32	Ad libitum	4.5, 5.2, 5.9, 6.6, 7.3	0.31, 0.35, 0.40, 0.45, 0.50	BW gain* Feed:gain* N-retention*	0.45	0.05	0.43	0.43	[3], castrated males
	34	About 10-15	13.7/190/87	Ad libitum	6.1, 6.5, 6.9, 7.3, 7.7	0.44, 0.47, 0.50, 0.53, 0.56	BW gain* Feed:gain* N-retention*	<0.44			<0.29	[3], castrated males
	35	About 10-15	13.5/184/87	Ad libitum	4.9, 5.4, 5.9, 6.4, 6.9	0.36, 0.40, 0.44, 0.47, 0.51	BW gain* Feed:gain N-retention*	0.47	0.03	0.46	0.31	[3], castrated males

Lenehan et al. (2003)	36	12-27	16.4/225/87	Ad libitum	9.0, 9.6, 10.2, 10.8, 11.5	0.55, 0.59, 0.62, 0.66, 0.70	Feed intake BW gain* Feed:gain* Plasma urea	0.62	0.03	0.61	0.56	[2]
Lenis and van Diepen (1990)	37	45-70	8.5/134/93	Ad libitum	4.5, 5.1, 5.7, 6.3	0.53, 0.60, 0.67, 0.74	Feed intake BW gain* Feed:gain*	0.67	0.07	0.64	0.59	females
	38	45-70	8.5/134/93	Ad libitum	4.5, 5.1, 5.7, 6.3	0.53, 0.60, 0.67, 0.74	Feed intake* BW gain* Feed:gain*	0.60	0.07	0.57	0.51	castrated males
	39	70-105	8.5/134/93	Ad libitum	4.5, 5.1, 5.7, 6.3	0.53, 0.60, 0.67, 0.74	Feed intake* BW gain* Feed:gain*	<0.53			<0.43	females
	40	70-105	8.5/134/93	Ad libitum	4.5, 5.1, 5.7, 6.3	0.53, 0.60, 0.67, 0.74	Feed intake* BW gain* Feed:gain*	<0.53			<0.43	castrated males
Lewis and Peo (1986)	41*	Mean initial weight was 6.4	14.3/178/82	Ad libitum	5.9, 6.4, 6.9, 7.6, 8.4, 9.3	0.41, 0.45, 0.48, 0.53, 0.59, 0.65	Feed intake BW gain Feed:gain* Plasma urea	0.53	0.05	0.51	0.48	[3]
Li and Xiao (1998)	42	17-30	9.6/180/149	Ad libitum	5.9, 6.9, 8.0, 9.1	0.61, 0.72, 0.83, 0.95	Feed intake BW gain* Feed:gain*	0.72	0.11	0.67	0.68	[2]
Malmlöf et al. (1994)	43	Mean initial weight was 35	8.9/135/105	Restricted (two times / day)	4.9, 5.5, 6.1, 6.6	0.55, 0.61, 0.68, 0.74	Plasma urea*	0.61	0.06	0.58	0.57	[3]
	44*	Mean initial weight was 35	11.2/135/105	Restricted (two times / day)	4.9, 5.5, 6.1, 6.6	0.44, 0.49, 0.54, 0.59	Plasma urea*	0.49	0.05	0.47	0.44	[3]
O'Connell et al. (2007)	45	35-60	10.0/162/110	Ad libitum	5.5, 5.7, 6.3, 6.6, 6.9	0.55, 0.57, 0.63, 0.66, 0.69	Feed intake BW gain* Feed:gain*	0.63	0.06	0.60	0.62	castrated males
	46	35-60	9.0/150/110	Ad libitum	5.0, 5.6, 5.9, 6.2, 6.4	0.56, 0.62, 0.66, 0.69, 0.71	Feed intake* BW gain* Feed:gain*	<0.56			<0.56	females
	47	80-100	10.0/160/112	Ad libitum	5.5, 6.0, 6.3, 6.7, 8.0	0.55, 0.60, 0.63, 0.67, 0.80	Feed intake BW gain Feed:gain*	0.60	0.05	0.58	0.61	castrated males
	48	80-100	8.0/132/110	Ad libitum	4.4, 4.8, 5.0, 5.3, 5.5	0.55, 0.60, 0.63, 0.66, 0.69	Feed intake BW gain* Feed:gain*	0.66	0.03	0.65	0.70	females

Pedersen et al. (2003)	49	70-100	8.2/161/104	Restricted (three times / day)	4.8, 5.1, 5.3, 5.6	0.58, 0.62, 0.65, 0.68	Plasma urea *	0.62	0.04	0.60	0.62	[2], 30 MJ ME/day
	50	70-100	8.2/161/104	Restricted (three times / day)	4.8, 5.1, 5.3, 5.6	0.58, 0.62, 0.65, 0.68	Plasma urea *	0.62	0.04	0.60	0.62	[2], 40 MJ ME/day
	51	60-103	8.2/161/104	Restricted (three times / day)	4.8, 5.1, 5.3, 5.6	0.58, 0.62, 0.65, 0.68	BW gain* Feed:gain*	<0.58			<0.58	[2], 30 MJ ME/day
	52	60-103	8.2/161/104	Restricted (three times / day)	4.8, 5.1, 5.3, 5.6	0.58, 0.62, 0.65, 0.68	BW gain Feed:gain*	0.62	0.04	0.60	0.62	[2], 40 MJ ME/day
	53	58-75	8.2/161/104	Restricted (three times / day)	4.8, 5.2, 5.6, 5.9	0.58, 0.63, 0.68, 0.72	N-retention*	<0.58			<0.58	[2], 25.8 MJ ME/day
	54	58-75	8.2/161/104	Restricted (three times / day)	4.8, 5.2, 5.6, 5.9	0.58, 0.63, 0.68, 0.72	N-retention*	<0.58			<0.58	[2], 34.4 MJ ME/day
Pozza et al. (2000)	55	15-30	10.3/186/87	Ad libitum	6.0, 6.3, 7.0, 7.6, 8.2	0.55, 0.61, 0.68, 0.74, 0.80	Feed intake BW gain Feed:gain Plasma urea*	0.68	0.07	0.65	0.67	[3], females
Rosell and Zimmerman (1985)	56	5-15	11.4/162/67	Ad libitum	5.5, 6.0, 6.5, 7.0, 7.5	0.48, 0.53, 0.57, 0.61, 0.66	Feed intake* BW gain Feed.gain Plasma urea*	0.61	0.04	0.59	0.46	[3], 0.40% added methionine to basal diet had no effect (Exp. 2+3)
Saldana et al. (1994)	57	6-16	13.8/205/62	Ad libitum	6.9, 7.2, 7.6, 8.1, 8.5	0.50, 0.52, 0.55, 0.59, 0.62	Feed intake BW gain Feed:gain*	> 0.62			>0.49	[3]
	58	58-96	7.9/118/62	Ad libitum	3.3, 4.0, 4.6, 5.1, 5.6	0.42, 0.51, 0.58, 0.65, 0.71	Feed intake BW gain* Feed:gain*	0.58	0.07	0.55	0.44	[3]
Schutte et al. (1990)	59	20-40	10.4/160/98	Ad libitum	5.6, 6.2, 6.8, 7.4	0.54, 0.60, 0.65, 0.71	Feed intake BW gain* Feed:gain*	0.65	0.05	0.63	0.59	
Schutte et al. (1997)	60	50-60	9.7/165/98	Ad libitum	5.4, 5.8, 6.3, 6.7	0.56, 0.60, 0.65, 0.70	Feed intake BW gain* Feed:gain*	0.65	0.05	0.63	0.57	
	61	50-60	10.2/170/86	Ad libitum	6.1, 6.5, 7.0, 7.4	0.59, 0.64, 0.68, 0.73	Feed intake BW gain* Feed:gain*	0.64	0.05	0.62	0.53	

	62	60-95	9.7/165/98	Ad libitum	5.4, 5.8, 6.3, 6.7	0.56, 0.60, 0.65, 0.70	Feed intake BW gain* Feed:gain	>0.70			>0.62	
	63	60-95	10.2/170/86	Ad libitum	6.1, 6.5, 7.0, 7.4	0.59, 0.64, 0.68, 0.73	Feed intake BW gain* Feed:gain*	0.64	0.05	0.62	0.54	
Taylor et al. (1982)	64*	25-55	9.5/120/101	Ad libitum	4.8, 5.2, 5.4, 5.6, 5.8, 6.0, 6.2, 6.6	0.51, 0.55, 0.57, 0.59, 0.61, 0.63, 0.65, 0.69	BW gain* Feed:gain* Plasma urea*	0.57	0.02	0.56	0.55	[3], females
Wang et al. (2006)	65	10-25	13.3/231/107	Ad libitum	6.5, 7.2, 8.0, 8.9, 10.0	0.49, 0.54, 0.60, 0.66, 0.75	Feed intake BW gain* Feed:gain Plasma urea Immunity	0.60	0.06	0.57	0.66	

Pcd.: precaecal digestible

If DM concentration of the diet is not given by the authors of the paper, DM is assumed to be 890 g/kg .

NDF concentrations of all experimental diets are calculated according to the feed table of CVB (2000).

[1] Experiments with lysine concentrations in crude protein higher than in ideal protein (diets with 7.6 – 8.5g/100 g CP) were excluded from the analysis (Table 2) and marked with *.

[2] Total threonine and lysine is calculated according to CVB (2000).

[3] Apparent pcd. threonine and lysine is calculated according to CVB (2000).

[4] The criterion used for deriving $T:L_{opt}$ is marked with *.

3.4 RESULTS

The $T:L_{opt}$ values of the 65 itemised experiments are shown in Table 3.1. In some experiments, lysine concentration in crude protein of the diets was high (7.6 – 8.5g/100 g CP) and above the value of the ideal protein, leading to an underestimation $T:L_{opt}$ since protein became the first-limiting factor. Therefore, experiment 1, 6, 7, 12, 13, 30, 31, 41, 44, and 64 (Table 3.1) had to be excluded from analysis. In further 15 experiments $T:L_{opt}$ could not be defined, because $T:L_{opt}$ was below the lowest T:L ratio tested or above the highest T:L ratio tested. These experiments were not considered as well; therefore, 40 experiments remained for final analysis. If BW gain is taken as the only criterion, $T:L_{opt}$ is detected correctly in 30 out of the 37 experiments (not related to the 40 experiments, because in three experiments plasma urea concentration was the only parameter tested). If feed:gain is used as only criterion, $T:L_{opt}$ is detected correctly in 33 experiments, and if both BW gain and feed:gain serve as criteria, in 35 experiments. In the residual two experiments feed intake as well as N-retention were the deciding criteria. The few experiments in which N balance, plasma urea concentration or immune function were recorded show that these parameters result in the same or lower $T:L_{opt}$, and, therefore they cannot be considered as more sensitively affected parameters than feed:gain ratio.

Table 3.2
Optimum threonine : lysine ratios ($T:L_{opt}$), lysine and NDF concentration in the diets, and body weight (BW) gain of pigs in the different studies (based on data of Table 3.1)

	No. of experiments	Mean	SD	Minimum	25 % percentile	Median	75 % percentile	Maximum
$T:L_{opt}$	40	0.61	0.070	0.44	0.51	0.62	0.68	0.74
Mean BW < 30 kg	16	0.58	0.094	0.44	0.46	0.61	0.69	0.74
Mean BW 30-70 kg	15	0.63	0.042	0.57	0.56	0.64	0.68	0.73
Mean BW > 70 kg	9	0.61	0.036	0.56	0.57	0.62	0.67	0.68
Difference between $T:L_{opt}$ and T:L below $T:L_{opt}$ ($T:L_{opt-1}$)	40	0.06	0.018	0.02	0.03	0.05	0.08	0.11
Mean of $T:L_{opt}$ and $T:L_{opt-1}$	40	0.59	0.072	0.38	0.48	0.60	0.66	0.71
Exp., in which $T:L_{opt}$ was below the T:L ratios tested	9	0.56	0.053	0.44		0.56		0.65
Exp., in which $T:L_{opt}$ was above the T:L ratios tested	6	0.67	0.035	0.62		0.68		0.70
Optimum apparent pcd. threonine : lysine ratio	40	0.57	0.098	0.31	0.43	0.59	0.69	0.72
Lysine concentrations of the diets (g/kg DM)	39	10.6	2.5	6.9	8.1	10.2	14.1	16.4
BW gain (kg/d) of animals at $T:L_{opt}$	37	0.73	0.26	0.14	0.36	0.73	1.03	1.17
NDF content of the diets (g/kg DM)	39	90	23.5	32	56	93	114	149

Exp.: Experiments

The overall mean for $T:L_{opt}$ is 0.61 (n = 40), and the minimum of 0.44 and the maximum of 0.74 reflect the high variation between experiments (Table 3.2). The split up into BW categories did not show any effect of BW on $T:L_{opt}$. The mean of the difference between $T:L_{opt}$ and the nearest T:L ratio tested below $T:L_{opt}$ ($T:L_{opt-1}$) is 0.06, showing the large steps of supplementation in the experiments, leading to the conclusion that $T:L_{opt}$ might be overestimated, because the true optimum ratio can lie between both ratios. The overall mean for pcd. $T:L_{opt}$ is 0.58 (n = 40), whereat the range is of similar magnitude as on total basis. The lysine level of the diets in the different experiments was pretty high, leading to BW gains of 0.73 kg/d in average; very low values (mean 0.18 kg/d) were received in studies with animals of 2 kg initial BW.

An attempt was made to ascertain possible factors affecting $T:L_{opt}$: BW (A), BW gain (B) and lysine concentration in the diet (D) as measures of the animal production level; protein concentration in the diet (C) as an indicator of potential imbalances of threonine to other AA; year of publication (E) as possible indicator of the genetic improvement of the animals; NDF concentration in the diet (F) as a possible anti-nutritive factor increasing endogenous protein losses or decreasing absorption of AA. The respective relationships are illustrated in Figure 3.1 (the capital letters are related to the text). BW, BW gain, and protein and lysine concentration did not seem to have any effect on the optimum ratio, while $T:L_{opt}$ increased with the year of publication ($p = 0.003$) and with the NDF concentration in the diet ($p < 0.001$). The regression equation between $T:L_{opt}$ (Y) and year of publication (X) is Y = 0.00405 (SE = 0.0013) X - 7.467 (SE = 2.53) (n = 40; $R^2 = 0.23$; RMSE = 0.065), and between $T:L_{opt}$ (Y) and NDF concentration in the diet (X) is Y = -0.0000115 (SE = 0.0000011) X^2 + 0.00435 (SE = 0.00031) X + 0.319 (SE = 0.028) (n = 40; $R^2 = 0.64$, RMSE = 0.045).

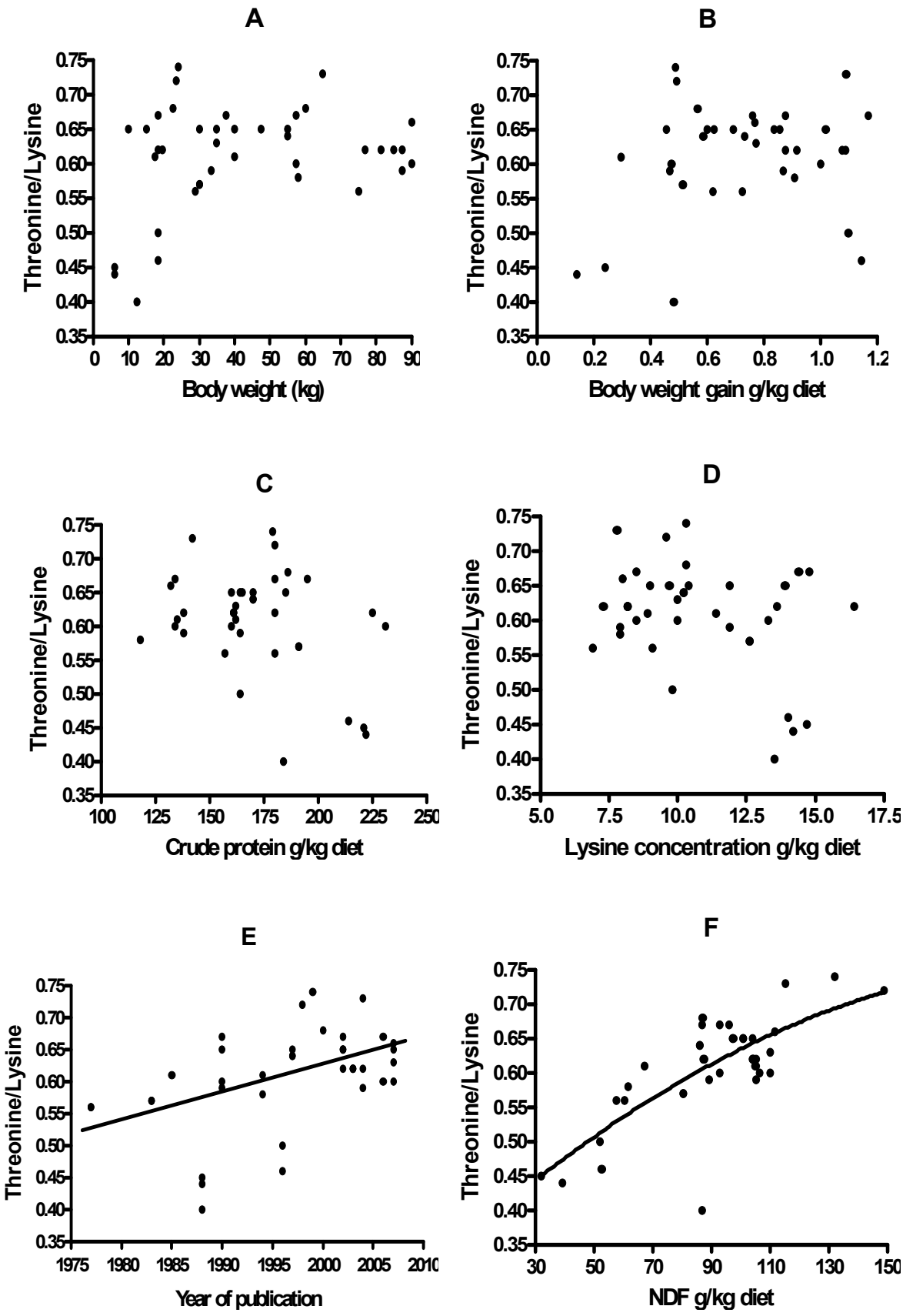

Figure 3.1 Optimum threonine : lysine ratio in diets for pigs as affected by body weight (A), body weight gain (B), crude protein concentration in the diet (C), lysine concentration in the diet (D), year of publications of the study (E), and NDF concentration in the diet (F). Data (n = 40) were taken from Table 1.

3.5 DISCUSSION

The overall mean value of 0.61 for T:L_{opt} cannot be taken as a general recommendation for diet formulation, because it should be higher to ensure an adequate supply for the majority of animals. Due to the high variation in T:L_{opt}, a general recommendation should add e.g. one SD to the mean to cover the requirement of 84% of the animals (T:L = 0.68). Furthermore, the mean is lower than the recommendations of GfE (2008) (T:L = 0,63 for a pig weighing 50 kg with a BW gain of 700 g/d). This low mean value might associate with the low fibre contents in most of the experimental diets; this aspect will be discussed below in more detail.

The mean of the nearest T:L ratio tested below T:L_{opt} (T:L_{opt-1}) is 0.55, therefore the difference (T:L_{opt} -T:L_{opt-1}) is quite high with 0.06. This fact leads to an uncertainty in the determination of the optimum ratio, because it may lie between both ratios, which means that the mean T:L_{opt}-value might overestimate the true optimum supply, but on the other hand would give additional safety when used for practical diet formulation. The reason for the lower optimum ratio in pcd. AA than in total AA could be that pc. digestibility of threonine is lower than that of lysine in most of the feed components used for the experimental diets (CVB, 2000). Henry (1983) demonstrated that threonine supply for maximal feed intake is lower than that for maximal growth, which is in agreement with the results of the present analysis, where parameters other than feed intake were the deciding criteria for T:L_{opt}. Also Cole et al. (1983) found that feed intake was affected by threonine intake however reached its maximum at a lower level than for growth.

Factors which may influence the requirement for AA are: age or BW of the animals, level of growth rate, genetic origin, gender, dietary factors (protein, energy or fibre content of the diet), and environmental conditions (e.g. health status and temperature) (Berende et al., 1983). Amongst others Pedersen et al. (2003) found that the dietary T:L_{opt} for pigs increases with BW, which could not be confirmed in the present study. Furthermore, the studies of e.g. Conway et al. (1990) and Pedersen et al. (2003) indicated that T:L_{opt} is not influenced by moderate variations in daily gain, which agrees with our evaluation of the data. As reported by Henry and Seve (1993) females have a higher protein and AA requirement than castrated males which might affect optimum AA ratios. Five experiments were found for which the optimum ratios were determined separately for females and castrated males. In two of these, T:L_{opt} was not affected by gender, in two the ratio in females was higher, and in one lower than in castrated males, consequently there are no consistent observations to draw a general conclusion. Furthermore, no correlation between T:L_{opt} and dietary protein concentration was detected (Figure 3.1. (C)), which does not confirm the observation of Thong and Liebert (2004) who found that dietary T:L_{opt} for pigs decreases with increasing protein concentration. The reason that T:L_{opt} is higher in more recent publications could be, that a correlation exists

between NDF concentration and year of publication (R^2 = 0.35; p < 0.001), indicating an increasing use of fibre rich feeds. Therefore, the effect of year of publication should not be interpreted. Very interesting is the effect of NDF concentration in the diet on the optimum ratio, which is affirmed by recent experiments of Blank et al. (2008), who showed that dietary fibre decreases the amount of threonine available for growth. This also agrees with observations of Schulze et al. (1994), who ascertained increasing protein ileal flow with increasing amounts of NDF in the diet. Increased losses of endogenous protein can be caused by increased secretion and/or decreased re-absorption (Schulze et. al., 1994). Furthermore, fibre may adsorb AA and withhold them from absorption, increases sloughing of intestinal mucosal cells, and enhances mucus production (Sauer et al., 1991). The mean NDF concentration in the diets of the 40 experiments is 90 g /kg DM, which is very low when compared to commercial diets containing frequently 150 g or even more NDF/kg DM. Figure 3.1 (F) illustrates the effect of NDF concentration in the diet on $T:L_{opt}$ and shows that $T:L_{opt}$ does not proportionally increase with increasing concentration of NDF. According to this equation $T:L_{opt}$ is 0.59, 0.64, and 0.67 at 80, 100, and 120 g NDF/kg DM. However, due to few observations an estimate for $T:L_{opt}$ at NDF concentrations higher than 120 g/kg does not seem to be adequate.

3.6 CONCLUSION

It is concluded from the results of this study that threonine requirement or optimum supply to pigs should not be defined without considering the NDF content of their diets. For commercial diets in the intensive pig production an optimum ratio between 0.65 – 0.69 might be appropriate. Further research seems to be necessary to determine the optimum ratio at different fibre concentrations.

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4 GENERAL CONCLUSIONS AND SUMMARY

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4.1 CONCLUSIONS

The validity of the alternative indirect approach applied in this study to determine the negative effect of dietary fibre on the amount of threonine available for protein retention could convincingly be confirmed. Therefore, this approach is superior above the classical approach where the amounts of endogenous protein and AA are determined at the terminal ileum in cannulated animals. The amount of endogenous protein or AA at the end of the terminal ileum does not reflect additional losses caused by the synthesis of the non- and re-absorbed endogenous protein. The experiments could show significant effects of dietary fibre on protein retention. Based on these data an estimate of the increased threonine requirement with increasing fibre concentration could be made. Furthermore, it was shown that the extent of these effects depend on fibre source, however are independent of the fibre concentration in the diet. Particle size and water-holding capacity seen as factors affecting EAAL (Dierick et al., 1989, and Souffrant, 2001) did not show any relationship to the findings in this study. Therefore, it is particularly interesting to carry out further experiments to identify the reasons for the different impacts of fibre and to test the effect of fibre degrading enzymes of which it is known to reduce EAAL.

The results of the experimental studies as well as of the literature review clearly show the need to consider diet composition as factor affecting threonine requirement in pigs. Since standard recommendations for AA supply were derived from studies using low fibre diets, it seems to be necessary to increase the recommended values for adequate threonine supply in commercial diets and to define $T:L_{opt}$ not as a constant ratio but to express this optimum as a function of fibre concentration in the diet.

4.2 SUMMARY

Dietary factors (e. g. fibre) which may increase endogenous protein/threonine losses, which reduce growth and protein retention of animals when certain amino acid are limiting factors, are usually not considered in feeding standards (GfE, 2006; NRC, 1998). The aim of the present studies was to give more precise information about the threonine requirement for maintenance which may lead to modifications of these recommendations.

In the first study the effect of different dietary fibres on endogenous protein/threonine losses in pigs was examined. However, the amounts of protein and amino acids of endogenous

origin appearing at the terminal ileum as determined in cannulated animals do not represent the total amount of losses associated with endogenous secretion. A high proportion of secreted protein is reabsorbed (up to 0.79) and does not reach the terminal ileum, and losses occur during synthesis of endogenous protein. Therefore, in the present study an alternative indirect approach was used, where the reduction of N retention in a threonine limiting diet was taken as a sensitive indicator for fibre associated threonine losses. Threonine is considered as first limiting amino acid in this study, because threonine concentration in endogenous protein is high and, therefore, a reduction of the amount of threonine available above maintenance will highly affect protein deposition. The extent of this effect may be dependent on the amount as well as on the source of fibre. Therefore, two experiments were conducted with twelve castrated male pigs each to measure the effect of threonine intake and the effect of 150 and 300 g/d fibre intake from wheat bran (WBF) (Exp.1), and of 150 g/d fibre from rape seed (RSF), cassava leaves (CLF), and cassava roots (CRF) (Exp. 2) on N retention, respectively. Generally, the results of the experiments showed that WBF and RSF reduced corrected N retentions ($P < 0.01$), whereas CLF and CRF did not show a significant negative effect. The reason for these observations that WBF and RSF caused larger effects than CLF and CRF could not be conclusively attributed. The higher amount of WBF (300) resulted in a further decrease of N retention when compared to WBF (150) ($P = 0.007$). Consequently, observations from this study show that corrected N retentions were affected by fibre level ($p = 0.007$) and source ($p < 0.001$). Fibre associated threonine losses amounted for 3.2, 3.3, 3.4, 1.2, and 1.1 g/kg WBF (150), WBF (300), RSF, CLF, and CRF, respectively. It can be concluded that threonine losses per g dietary fibre depend on the fibre source and are not affected by their concentration in the diet.

In the second study all available data reported in the literature are compiled which determined and evaluated the optimum threonine : lysine ratio ($T:L_{opt}$) in the diets of growing pigs. There were found 65 experiments, published between 1977 and 2007, of which in 15 experiments $T:L_{opt}$ could not be defined, because in these experiments $T:L_{opt}$ was below the lowest T:L ratio tested or above the highest T:L ratio tested. Only experiments were included in which threonine was the first limiting amino acid and lysine the second-limiting nutrient, therefore, further 10 experiments could not be considered. The experimental parameters recorded were in nearly all studies feed intake, body weight gain and feed conversion rate, and in some studies N balance, plasma urea concentrations and immunity. To ensure that the requirement was satisfied, the response criteria with the highest value of $T:L_{opt}$ was selected. The overall mean for $T:L_{opt}$ was 0.61 ($n = 40$), while the overall mean for pcd. $T:L_{opt}$ was 0.57 ($n = 40$). Based on these 40 experiments an attempt was made to ascertain the factors affecting the $T:L_{opt}$. Initial body weight, body weight gain, and lysine and protein concentration in the diet did not seem to have any effect on the optimum ratio, while year of

publication ($p = 0.003$) and NDF concentration in the diet ($p < 0.001$) had a significant effect. It can be concluded from both studies that dietary fibre increases threonine requirement and $T:L_{opt}$ in the diets for growing pigs. Therefore, recommendations for amino acid supply should consider these specific effects of diet composition.

4.3 ZUSAMMENFASSUNG

Verschiedene Einflussfaktoren der Diät (z.B. Faser), welche die endogenen Protein-/Threoninverluste erhöhen und das Wachstum und den Proteinansatz des Tieres reduzieren, wenn bestimmte Aminosäuren limitierende Faktoren sind, werden üblicherweise in den internationalen Fütterungsempfehlungen (GfE, 2006; NRC, 1998) nicht berücksichtigt. Das Ziel der vorliegenden Studien war es, derartige Einflüsse auf den Erhaltungsbedarf für Threonin zu untersuchen.

Der Grund für die erste Studie war der in der Literatur beschriebene Effekt, dass Nahrungsfaser die endogenen Protein- und Aminosäureverluste erhöht. Die Menge an Protein und Aminosäuren endogener Herkunft, welche am Ende des Ileums fistulierter Tieren ermittelt wird, repräsentiert jedoch nicht die gesamte Menge an den mit der endogener Sekretion verbundenen Verluste. Ein großer Anteil des abgesonderten Proteins wird rückabsorbiert (bis zu 0.79) und erreicht das Ende des Ileums nicht, so dass bei dieser Messung die Verluste, die bei der Synthese des endogenen Proteins auftreten, nicht berücksichtigt werden. Daher wurde in der vorliegenden Studie ein alternativer indirekter Ansatz gewählt, bei welchem die Reduktion der N Retention in einer Threonin limitierten Diät als ein sensibler Indikator für die mit Faser verbundenen Verluste genutzt wurde. Als erstlimitierende Aminosäure ist Threonin gewählt worden, da die Threoninkonzentration im endogenen Protein hoch ist, und eine Reduktion des oberhalb des Erhaltungsbedarfs verfügbaren Threonins sich somit deutlich auf den Proteinansatz auswirkt. Das Ausmaß des Effekts kann von der Fasermenge sowie der Faserquelle abhängig sein. Daher wurden zwei Experimente mit jeweils zwölf Börgen durchgeführt, in denen der Effekt der Threonin-aufnahme sowie der Effekt von 150 and 300 g/Tag Faseraufnahme aus Weizenkleie (WBF) (Exp.1) und jeweils 150 g/Tag Faser aus Rapsextraktionsschrot (RSF), Cassava Blättern (CLF) und Cassava Wurzelresten (CRF) (Exp.2) bestimmt wurde. Die Ergebnisse der Experimente zeigten, dass WBF und RSF die N Retention reduzierten ($P < 0.01$), wogegen CLF and CRF keinen signifikanten negativen Effekt zeigten. Der Grund für diese unterschiedlichen Effekte konnte in dieser Studie jedoch nicht gefunden werden. Die höhere Menge an WBF (300) resultierte in einer weiteren Abnahme der N Retention im Vergleich zur WBF (150) ($P = 0.007$). Folglich zeigten die Beobachtungen dieser Studie, dass die N

Retention durch die Faserhöhe ($p = 0.007$) sowie Faserquelle ($p < 0.001$) beeinflusst wird. Die mit Faser verbundenen Verluste betrugen für jeweils WBF (150), WBF (300), RSF, CLF und CRF 3.2, 3.3, 3.4, 1.2 und 1.1 g/kg. Somit kann geschlussfolgert werden, dass die Threoninverluste pro g Nahrungsfaser von der Faserquelle abhängig sind und nicht von ihrer Konzentration in der Diät beeinflusst werden.

In der zweiten Studie wurde die gesamte verfügbare Literatur zur Frage des optimalen Threonin-Lysin-Verhältnisses ($T:L_{opt}$) in Rationen für wachsende Schweine ausgewertet. Es wurden 65 Experimente gefunden, die zwischen 1977 und 2007 veröffentlicht worden sind. In 15 Experimenten konnte $T:L_{opt}$ nicht bestimmt werden, weil $T:L_{opt}$ in diesen Experimenten entweder unterhalb des niedrigsten oder über dem höchsten getesteten T:L Verhältnis lag. Es wurden nur Experimente berücksichtigt, in denen Threonin die erstlimitierende und Lysin die zweitlimitierende Aminosäure war, somit blieben 10 weitere Experimente unberücksichtigt. Die experimentellen Parameter waren in fast allen Studien Futteraufnahme, Gewichtszuwachs und Futteraufwand, in einigen Studien N Bilanz, Blut-Harnstoff Konzentration und Immunstatus. Um abzusichern, dass der Bedarf gedeckt ist, wurde der Parameter mit dem höchsten Wert für $T:L_{opt}$ für die weitere Auswertung verwendet. Der Mittelwert für $T:L_{opt}$ betrug 0.61 ($n = 40$), während der Mittelwert für das verdauliche $T:L_{opt}$ mit 0.57 ($n = 40$) niedriger war. Es wurde der Versuch unternommen Faktoren zu ermitteln, welche $T:L_{opt}$ beeinflussen. Körpergewicht, Gewichtszuwachs sowie Lysin- und Proteinkonzentration in der Diät schienen keinen Effekt auf das optimale Verhältnis zu haben, während die NDF Konzentration in der Diät einen signifikanten Effekt zeigte. Es kann daher aus den beiden Studien gefolgert werden, dass der Fasergehalt einen Einfluss auf den Threoninbedarf ausübt und damit $T:L_{opt}$ in der Ration vom Fasergehalt abhängt.

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